

BB 1x showed that the OmpT<sup>+</sup> cells can be readily isolated by FACS from a huge excess of background cells solely on the basis of the enzymatic activity of the OmpT protease FIGS. 11A-11C.

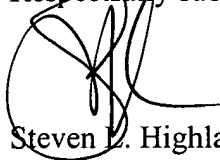
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### REMARKS

The instant preliminary amendment is provided to delete reference to original FIGS. 11A and 11B, which apparently were not submitted to the U.S. Patent and Trademark Office with applicants' priority filing U.S. Serial No. 08/847,063. The examiner is invited to contact the undersigned with any questions regarding this paper.

Please date stamp and return the accompanying postcard to evidence receipt of these documents.

Respectfully submitted,



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Date: July 3, 2001

## APPENDIX A: MARKED UP COPY OF THE SPECIFICATION

At page 11, delete the paragraphs at lines 7-16:

[**FIG. 11A and FIG. 11B:** FIG. 11A shows fluorescence histograms of cells expressing inactive OmpT after exposure to the fluorogenic substrate. FIG. 11B shows the flow cytometric fluorescence of cells expressing wild-type (active) OmpT) after the exposure to the fluorogenic substrate.]

[**FIG. 12A-12C**] FIG. 11A-11C: [FIG. 12A] FIG. 11A shows a fluorescence histogram of 20,000 cells from a mixture of OmpT<sup>+</sup> and OmpT<sup>-</sup> at a ratio of 1:5,000. After sorting, 32 cells were collected and the fluorescence of nine clones was examined by FACS. [FIG. 12B] FIG. 11B and [FIG. 12C] FIG. 11C show representative fluorescence histograms for two of the isolated OmpT<sup>+</sup> clones.

At pages 91-92, delete the paragraph bridging the pages:

Different strains of bacteria were exposed to the substrate for 10 min. and examined by FACS. The OmpT<sup>-</sup> negative *E. coli* mutant UT5600, shows no fluorescence [(FIG. 11A)]. However, UT5600 cells expressing OmpT from a multicopy plasmid (pML19), showed a much larger increase in fluorescence, which continued to increase for over 20 minutes. The mean fluorescence intensity of OmpT<sup>+</sup> cells was over 30 times higher than that of the cells without the plasmid (*i.e.*, OmpT<sup>-</sup> cells). Such a difference in OmpT fluorescence is more than sufficient to allow the sorting of cells expressing active enzyme from cells that do not express OmpT [(FIG. 11)].

At page 92, the paragraph at lines 16-23:

In other studies it was demonstrated that OmpT<sup>+</sup> cells can readily be isolated from a population containing a huge excess of OmpT<sup>-</sup> cells. Specifically, OmpT<sup>+</sup> cells were mixed with OmpT<sup>-</sup> cells at a 5,000-fold excess. The mixture was incubated with the substrate, passed through the fluorescence activated cell sorter and cells exhibiting a high fluorescence intensity were isolated. Nine out of nine sorted clones that were isolated produce OmpT. These studies

showed that the OmpT<sup>+</sup> cells can be readily isolated by FACS from a huge excess of background cells solely on the basis of the enzymatic activity of the OmpT protease [(FIG. 12)] FIGS. 11A-11C.